Analgesic effect of intrathecal desipramine on carrageenan-induced thermal hyperalgesia in the rat

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We examined if intrathecal desipramine, a selective norepinephrine reuptake inhibitor, would modulate peripheral inflammation-induced hyperalgesia. Rats were chronically implanted with a lumbar intrathecal catheter and paw withdrawal latency (PWL) to noxious heat stimuli was assessed. Unilateral hindpaw inflammation was induced by intraplantar carrageenan injection. Carrageenan injection significantly \((P<0.05)\) reduced PWL of the injected paw (from mean 11.4 (SEM 0.6) s to 3.5 (0.2) s, 3 h after carrageenan), but not of the contralateral side (from 11.6 (0.2) s to 11.2 (0.5) s). Intrathecal desipramine 10, 30, 60 and 100 \(\mu\)g, which did not produce analgesic effects in untreated rats, dose-dependently reversed the shortened PWL on the ipsilateral side (3.3 (0.2), 5.3 (0.4), 6.2 (0.3) and 9.6 (0.2) s, respectively) without affecting the contralateral side. Pretreatment with intrathecal yohimbine 10 \(\mu\)g did not antagonize the anti-hyperalgesic effects of desipramine (from 9.6 (0.2) to 9.8 (0.3) s). Our results suggest that the mechanism underlying the analgesic effect of desipramine on inflammation-induced hyperalgesia is unlikely to be inhibition of norepinephrine reuptake within the spinal cord.

Keywords: pain, experimental; pain, model; pharmacology, desipramine; rat

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Tricyclic antidepressants are often administered systemically to patients with chronic pain as co-analgesics, and are known to show modest efficacy.\(^1\) In addition, it is reported that oral desipramine enhanced the analgesic effect of i.v. morphine for postoperative pain.\(^2\) Animal studies have reported that intrathecal administration of desipramine potentiated morphine analgesia as a result of possible selective inhibition of uptake of norepinephrine in spinal nerve terminals.\(^3\) \(^4\) However, in patients with depressive disorders, the analgesic responses of tricyclic antidepressants frequently occur more rapidly and at lower doses than typical antidepressant responses.\(^5\) In addition, a structure–activity analysis failed to demonstrate a clear relationship between specific efficacy for inhibition of either norepinephrine or serotonin reuptake and analgesia.\(^1\) Thus it seems that other mechanisms also contribute to their analgesic actions. Recent in vitro studies have demonstrated that desipramine and other tricyclic antidepressants have an antagonistic action at the N-methyl-D-aspartate (NMDA) receptor.\(^5\) \(^9\) Therefore, intrathecal desipramine may modulate pain transmission via NMDA receptor antagonism and also via activation of the noradrenergic system.

In this study, we have examined, in rats, if intrathecal desipramine modulated peripheral inflammation-induced hyperalgesia, which has been shown to have a spinal NMDA receptor component. 

Materials and methods

The study was approved by Sapporo Medical University Animal Care and Use Committee. Experiments were conducted in male Sprague–Dawley rats (weighing 250–300 g, Japan SLC, Hamamatsu, Japan), which were housed individually in a temperature-controlled (21±1°C) room with a 12-h light–dark cycle and given free access to food and water.

Animal preparation and surgical procedure

Under general anaesthesia (2% isoflurane in oxygen), a polyethylene intrathecal catheter (PE-10, Clay Adams, NJ, USA) was inserted 15 mm cephalad into the lumbar subarachnoid space at the L4–5 intervertebrae with the tip of the catheter located near the lumbar enlargement of the spinal cord, using a method described previously.\(^10\) At least 6 days were allowed after surgery for recovery of animals. In the experiments, we used only animals that showed normal behaviour and motor function, which were assessed using the scale of Penning and Yaksh.\(^11\) We also confirmed that all animals had complete paralysis of the tail and
bilateral hind legs after administration of 2% lidocaine 10 µl via the intrathecal catheter.

**Unilateral inflammation**

Lambda carrageenan 2 mg (Sigma Chemical, St Louis, MO, USA) was injected s.c. via a 27-gauge needle into the plantar surface of the left hind paw under 1% isoflurane anaesthesia. Lambda carrageenan was suspended in normal saline by sonication and administered in a volume of 0.1 ml. After recovery from isoflurane anaesthesia, the animal was placed in a plexiglass box, allowing observation.

**Nociceptive testing**

Thermal nociceptive testing was conducted using an analgesimeter (Plantar Test 7370, Ugo Basile, Italy). Radiant heat was applied on the plantar surface of each hind paw. The thermal nociceptive threshold was evaluated as paw withdrawal latency (PWL) from the heat source. Bulb intensity was adjusted so that baseline PWL was 10–12 s.

**Drugs**

Intrathecal drug administration was accomplished using a microinjection syringe (Hamilton, Remo, NV, USA) connected to an intrathecal catheter in awake, briefly restrained rats. Desipramine (Sigma Chemical) was dissolved in dimethyl sulphoxide (DMSO) and subsequently diluted with physiological saline to 4% DMSO. Yohimbine (Sigma Chemical) was dissolved in physiological saline. The drugs were administered manually over a 10-s period in a single injection volume of 10 µl followed by a flush of physiological saline 15 µl.

**Effect of desipramine on thermal nociceptive testing and general behaviour**

To examine the effect of desipramine on PWL to thermal nociception and on general behaviour, desipramine or 4% DMSO was administered intrathecally. PWL of both sides was measured 10, 20, 30, 45 and 60 min after intrathecal administration. General behaviour was also evaluated at each time during nociceptive testing by a scoring system of two specific behaviours (normal or mild to severely impaired): (1) placing/stepping reflex—this response was evoked by drawing the dorsum of either hind paw across the edge of the table. This stimulus elicits an upward lifting of the paw from the surface of the table (stepping); (2) righting reflex—a rat placed horizontally with its back on the table will normally show an immediate coordinated twisting of the body around its longitudinal axis to regain its normal posture.

**Effect of desipramine on thermal hyperalgesia**

The experiments were designed to investigate the effects of intrathecal desipramine on PWL after thermal hyperalgesia was established. After basal PWL was obtained, carrageenan 2 mg (0.1 ml) was injected s.c. Consistent with previous reports, our preliminary studies revealed that maximum shortening of PWL was sustained for 3–6 h after injection of carrageenan. Accordingly, 3 h after carrageenan injection, rats received intrathecal desipramine or 4% DMSO. After this, PWL was measured at 10, 20, 30, 45 and 60 min.

In addition, to test if the effects of desipramine were mediated via the noradrenergic system within the spinal cord, some rats received intrathecal saline or yohimbine 10 µg, 10 min before administration of desipramine.

**Statistical analyses**

All data are presented as mean (SEM). Changes in PWL were analysed using one-way analysis of variance for repeated measures followed by Scheffé’s F test within a single group. *P*<0.05 was considered statistically significant.

**Results**

**Effect of desipramine on thermal nociceptive testing and general behaviour**

Baseline PWL values for the right and left paws of untreated rats were 10.9 (0.5) s and 11.1 (0.5) s, respectively. Intrathecal injection of desipramine 10, 30, 60 and 100 µg did not produce prolongation of PWL (data not shown). In addition, intrathecal injection of desipramine 10, 30 and 60 µg had no effect on placing, stepping or righting reflexes (data not shown). Although at a dose of 100 µg there were mildly impaired placing, stepping or righting reflexes temporarily in four of six rats that recovered fully within 10 min after injection, they were able to ambulate and were tested for thermal nociceptive threshold. Thus desipramine 100 µg was the highest dose used in this study.

**Effect of desipramine on thermal hyperalgesia**

Before injection of carrageenan, mean PWL values for the right and left paws were 11.2 (0.5) s and 11.4 (0.6) s, respectively. Carrageenan injection significantly reduced PWL on the injected side but not on the contralateral side, 1 h after injection and thereafter (Fig. 1).

Intrathecal desipramine, which was administered 3 h after carrageenan, reversed the shortened PWL on the ipsilateral side in a dose-dependent manner, but had no effect on the contralateral side (Fig. 2). The peak effects of desipramine were observed 10 min after intrathecal injection (Fig. 3). Intrathecal 4% DMSO had no effect on PWL on either side (data not shown). Pretreatment with intrathecal yohimbine 10 µg did not alter baseline PWL or the effects of desipramine 100 µg in carrageenan-treated rats (Fig. 3).

**Discussion**

We have shown that the tricyclic antidepressant desipramine, administered intrathecally, reversed thermal hyperalgesia in
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Fig 1 Effects of injection of carrageenan on paw withdrawal latency in carrageenan-treated and untreated paws. Each value represents mean (SEM); n=6. *P<0.05 compared with before injection of carrageenan (time 0).

Fig 2 Dose–response curves for intrathecal desipramine on paw withdrawal latency in carrageenan-treated and untreated paws. Data represent paw withdrawal latencies 10 min after intrathecal administration of desipramine. Each value represents mean (SEM); n=6 for each dose.

Fig 3 Effects of intrathecal desipramine 100 µg alone (□, ■) and desipramine 100 µg with yohimbine 10 µg pretreatment (○, ●) on paw withdrawal latency. Yohimbine was administered intrathecally 10 min before desipramine (upper arrow). Carrageenan-injected paws are shown as closed symbols and untreated paws as open symbols. Each value represents mean (SEM); n=6 for the yohimbine and saline groups. *P<0.05 compared with time 0.

half of the spinal cord in the model of chronic constriction injury of the sciatic nerve (CCI) increased, and that intrathecal yohimbine enhanced CCI-induced thermal hyperalgesia. In contrast to the CCI model, intrathecal yohimbine did not affect the thermal threshold in our study. We used yohimbine 10 µg which antagonized the antinociceptive effect of clonidine and enhanced thermal hyperalgesia in CCI rats, but had no effect on the thermal threshold in uninjured rats.15 16 This may suggest that the noradrenergic inhibitory system in the spinal cord is not activated in carrageenan-induced thermal hyperalgesia, but did not have any analgesic effects in untreated rats. In addition, the α2-adrenergic antagonist yohimbine, did not antagonize the anti-hyperalgesic effects of desipramine.

While the antinociceptive effect of desipramine in acute nociception is controversial,2 3 13 intrathecal desipramine enhances the antinociceptive effects of systemic morphine,2 3 which is known to activate a descending inhibitory pathway, including spinally projecting neurons, and to release norepinephrine and serotonin in the spinal cord.14 This potentiation of the antinociception of morphine was reversed by intrathecal yohimbine.2 Thus a possible mechanism of enhancement of intrathecal desipramine is that it blocks uptake of norepinephrine in spinal nerve terminals, resulting in potentiation of morphine analgesia.2 3 In our study, however, desipramine at the doses used did not produce antinociceptive effects in untreated rats.

In a model of peripheral nerve injury, the descending noradrenergic inhibitory system is activated. Satoh and Omote15 reported that norepinephrine content in the dorsal rats with carrageenan-induced peripheral inflammation, but did not have any analgesic effects in untreated rats. In addition, the α2-adrenergic antagonist yohimbine, did not antagonize the anti-hyperalgesic effects of desipramine.

One possible mechanism of the anti-hyperalgesic effect of intrathecal desipramine is antagonism of the NMDA receptor. Several lines of evidence have shown an NMDA receptor antagonistic property of desipramine.6–9 In vitro studies have suggested that desipramine binds either to the recognition site for Zn2+ or to the phencyclidine binding site within the cation channel.6 8 In vivo, Mjellen and colleagues12 showed that intrathecal desipramine reduced NMDA-induced behaviour, such as biting and scratching, in a dose-dependent manner. On the other hand, intraplantar carrageenan injection activated the NMDA receptor in the spinal cord, resulting in thermal hyperalgesia on the ipsilateral side.16–18 Furthermore, intrathecal NMDA antagonists, AP5 and MK-801, abolished thermal hyperalgesia...
with no effect on the contralateral side.\(^{17-19}\) Thus it is likely that the anti-hyperalgesic effects of intrathecal desipramine we observed were caused by antagonism of the NMDA receptor in the spinal cord. Previous studies have shown that intrathecal amitriptyline, another tricyclic antidepressant, also reverses carrageenan-induced hyperalgesia by a mechanism unrelated to inhibition of monoamine reuptake and inhibits intrathecal NMDA-induced behaviour.\(^{20}\)

In summary, we have examined the analgesic effects of desipramine at the level of the spinal cord. Intrathecal desipramine reversed thermal hyperalgesia in rats receiving intraplantar carrageenan, while it did not produce any analgesic effects in untreated rats. The mechanism of the anti-hyperalgesia is unlikely to be inhibition of norepinephrine reuptake.

References

8. Sills MA, Loo PS. Tricyclic antidepressants and dextromethorphan bind with higher affinity to the phencyclidine receptor in the absence of magnesium and L-glutamate. Mol Pharmacol 1989; 36: 160–5
10. Omote K, Sonoda H, Kawamata M, Namiki A. Potentiation of antinociceptive effects of morphine by calcium-channel blockers at the level of spinal cord. Anesthesiology 1993; 72: 746–52
19. Yamamoto T, Shimoyama N, Mizuguchi T. The effects of morphine, MK-801, an NMDA antagonist, and CP-96345, an NK1 antagonist, on the hyperesthesia evoked by carrageenan injection in the rat paw. Anesthesiology 1993; 78: 124–33
20. Eisenach JC, Gebhart GF. Intrathecal amitriptyline acts as an N-methyl-D-aspartate receptor antagonist in the presence of inflammatory hyperalgesia in rats. Anesthesiology 1995; 83: 1046–54